PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference JC18679/142	FOR FURTHER ACTION	See Form PCT/IPEA/416
International application No. PCT/NZ2004/000275	International filing date *(day/month/year)* 29 October 2004	Priority date *(day/month/year)* 31 October 2003

International Patent Classification (IPC) or national classification and IPC

Int. Cl.

See supplemental sheet

Applicant

AUCKLAND UNISERVICES LIMITED et al

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 7 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. [X] *(sent to the applicant and to the International Bureau)* a total of 22 sheets, as follows:
      - [X] sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      - [ ] sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.

   b. [ ] *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or table related thereto, in electronic form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).
4. This report contains indications relating to the following items:

[X]	Box No. I	Basis of the report
[ ]	Box No. II	Priority
[X]	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
[ ]	Box No. IV	Lack of unity of invention
[X]	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
[X]	Box No. VI	Certain documents cited
[ ]	Box No. VII	Certain defects in the international application
[X]	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
4 July 2005	09 February 2006
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized Officer IAN DOWD Telephone No. (02) 6283 2273

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/NZ2004/000275

Box No. I Basis of the report

1. With regard to the language, this report is based on:

[X] The international application in the language in which it was filed

[ ] A translation of the international application into , which is the language of a translation furnished for the purposes of:

[ ] international search (under Rules 12.3(a) and 23.1 (b))

[ ] publication of the international application (under Rule 12.4(a))

[ ] international preliminary examination (Rules 55.2(a) and/or 55.3(a))

2. With regard to the elements of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

[ ] the international application as originally filed/furnished

[X] the description:

pages **1-6, 9-11, 13-15, 18-63, 65** as originally filed/furnished

pages* **7-8a, 12, 16-17, 64** received by this Authority on **4 July 2005** with the letter of 1 July 2005

pages* received by this Authority on with the letter of

[X] the claims:

pages as originally filed/furnished

pages* as amended (together with any statement) under Article 19

pages* **66-80** received by this Authority on **4 July 2005** with the letter of 1 July 2005

pages* received by this Authority on with the letter of

[ ] the drawings:

pages as originally filed/furnished

pages* received by this Authority on with the letter of

pages* received by this Authority on with the letter of

[ ] a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing.

3. [ ] The amendments have resulted in the cancellation of:

[ ] the description, pages

[ ] the claims, Nos.

[ ] the drawings, sheets/figs

[ ] the sequence listing *(specify)*:

[ ] any table(s) related to the sequence listing *(specify)*:

4. [ ] This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

[ ] the description, pages

[ ] the claims, Nos.

[ ] the drawings, sheets/figs

[ ] the sequence listing *(specify)*:

[ ] any table(s) related to the sequence listing *(specify)*:

* *If item 4 applies, some or all of those sheets may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/NZ2004/000275

Box No. III **Non-establishment of opinion with regard to novelty, inventive step and industrial applicability**

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application

☒ claims Nos: **All claims (in part)**

because:

☐ the said international application, or the said claims Nos.
relate to the following subject matter which does not require an international preliminary examination *(specify)*:

☒ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. **all claims (in part)**
are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos.
are so inadequately supported by the description that no meaningful opinion could be formed *(specify)*

☐ no international search report has been established for said claim Nos.

☐ A meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit:

☐ Furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Preliminary Examining Authority in a form and manner acceptable to it.

☐ Furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Preliminary Examining Authority in a form and manner acceptable to it.

☐ Pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rules 13*ter*.1(a) or (b) and 13*ter*.2.

☐ A meaningful opinion could not be formed without the tables related to the sequence listings; the applicant did not, within the prescribed time limit, furnish such tables in electronic form complying with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions, and such tables were not available to the International Preliminary Examining Authority in a form and manner acceptable to it

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in electronic form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See Supplemental Box for further details.

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/NZ2004/000275

Box No. V **Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims **1-50**	**YES**
	Claims	**NO**
Inventive step (IS)	Claims **1-26**	**YES**
	Claims **27-50**	**NO**
Industrial applicability (IA)	Claims **1-50**	**YES**
	Claims	**NO**

2. Citations and explanations (Rule 70.7)

The following documents were cited in the International Search Report:

D1 Abstract 141:106328

D2 WO 2004/033415

D3 WO 2000/013683

D4 WO 1993/011099

D5 Abstract 137:134431

D6 Abstract 126:246358

D7 Abstract 125:753

D8 Abstract 123:275328

D9 Abstract 76:54221

D1 was published before the international filing date of the present application, but later than the priority date claimed. Under PCT guidelines, this document is excluded from consideration during international preliminary examination, however D1 is nevertheless included here for the purpose of information. This is based on the assumption that the claimed priority date is valid. Should this date subsequently be found invalid, then D1 may become relevant during national examination.

The present invention relates to nitrophenyl phosphate and nitrophenyl alcohol derivatives of formulae (I) and (II) respectively. These compounds are cytotoxic agents and are used in gene-directed enzyme-prodrug therapy (GDEPT) and antibody-directed enzyme-prodrug therapy (ADEPT).

Novelty and Inventive Step

D3-D9 all disclose nitrophenyl alcohol derivatives corresponding to the present formula (II) (relevant passages as indicated in the search report). These compounds have cytotoxic properties and are used in various forms of therapy, including GDEPT and ADEPT. Due to the provisos in claims 27 and 28, D3-D9 are now considered to be novel.

(Continued in Supplemental Box)

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/NZ2004/000275

Box No. VI Certain documents cited

1. Certain published documents (Rule 70.10)

Application No. Patent No.	Publication date (day/month/year)	Filing date (day/month/year)	Priority date (valid claim) (day/month/year)
WO 2004/033415 (D2)	22 April 2004	8 October 2003	8 October 2002

The above citation discloses nitrophenyl alcohol derivatives and their use in GDEPT. This document would be novelty destroying for claims 27-50 had it been published before the priority date of the present application.

Under PCT guidelines, novelty is only considered in respect of documents published before the priority date. The relevance of a document published after the priority date is dependent upon national law. Hence WO 2004/033415 may become relevant during national examination.

2. Non-written disclosures (Rule 70.9)

Kind of non-written disclosure	Date of non-written disclosure (day/month/year)	Date of written disclosure referring to non-written disclosure (day/month/year)

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

1. Claims 1 and 27, and their dependent claims, are not fully supported by the description. The claims define a broad range of compounds corresponding to formulae (I) or (II), however the description only provides an enabling disclosure for certain nitrophenyl derivatives. In particular, there is only support for compounds wherein X represents CONH and group Y is para to the nitro substituent on the phenyl ring.

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of: International Classification

C07C 233/67 (2006.01) *A61K 31/661* (2006.01) *C07C 317/36* (2006.01)
A61K 31/166 (2006.01) *A61P 35/00* (2006.01) *C07D 203/14* (2006.01)
A61K 31/396 (2006.01) *C07C 301/00* (2006.01) *C07F 9/09* (2006.01)

Continuation of: Box V

None of D3-D9 describe phosphate derivatives encompassed by the present formula (I). Claims 1-26 are therefore considered novel and inventive.

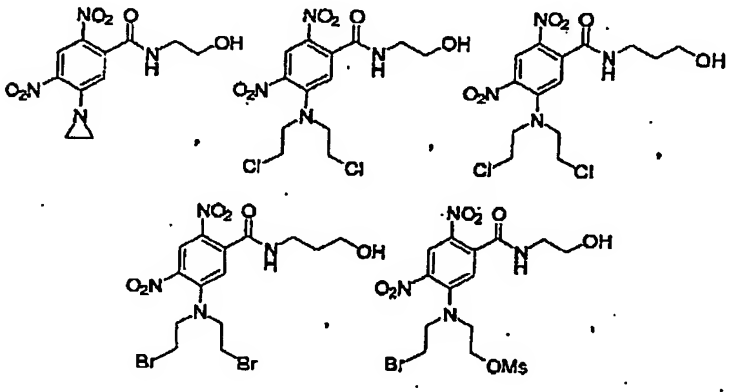
With regard to inventive step, the problem to be overcome is to provide improved targeted cytotoxic agents. The application addresses this need by providing nitrophenyl mustard and/or nitrophenylaziridine compounds. The prior art also addresses the same problem by providing nitrophenyl mustard and/or nitrophenylaziridine compound derivatives, where the only difference with the application lies in the exclusions as indicated in the amended claims. An inventive step can not therefore be acknowledged since it would be obvious to the person skilled in the art to utilise routine investigation to arrive at the compounds currently claimed. Furthermore there is no indication that all compounds claimed possess an improvement in cytotoxic activity over those of the prior art.

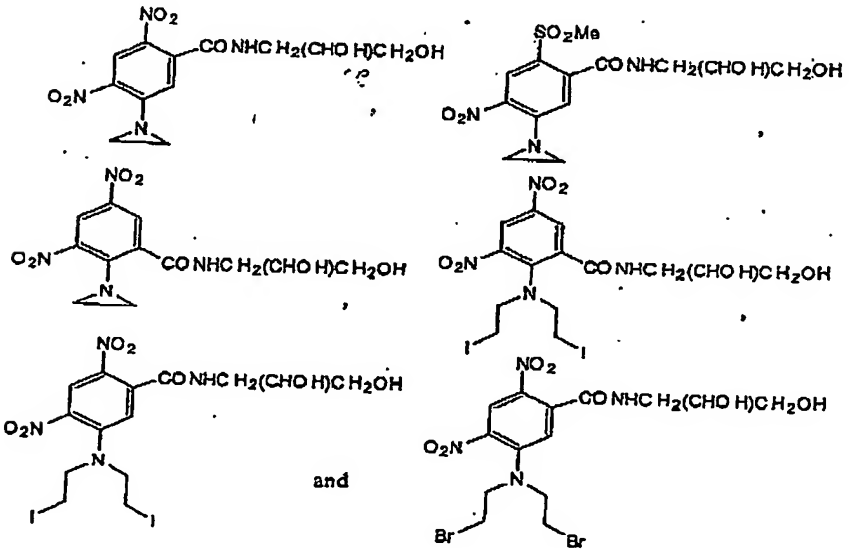
(See also Box VI, 'Certain documents cited')

Industrial Applicability

Claims 1-50 meet the requirements for industrial applicability.

R represents a lower C_{1-6} alkyl optionally substituted with one or more groups including hydroxy, amino and N-oxides therefrom or dialkylamino and N-oxides therefrom; and pharmaceutically acceptable salts and derivatives thereof, with the proviso that when Z represents NO_2 and Y represents $N(CH_2CH_2Cl)_2$, X and R together cannot represent $-CONHCH_2(CHOH)CH_2-$ and with the further proviso that the following compounds

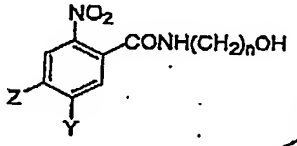




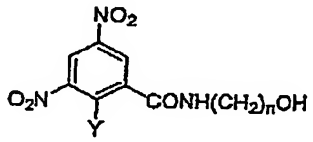
and

are excluded.

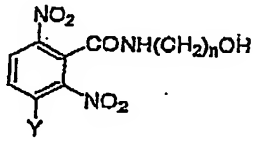
In a preferred embodiment, the alcohol compound of Formula (II) is selected from a compound represented by formulae (IIa), (IIb) or (IIc)



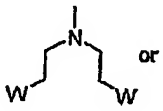
(IIa)



(IIb)



(IIc)

wherein Y may represent  or 

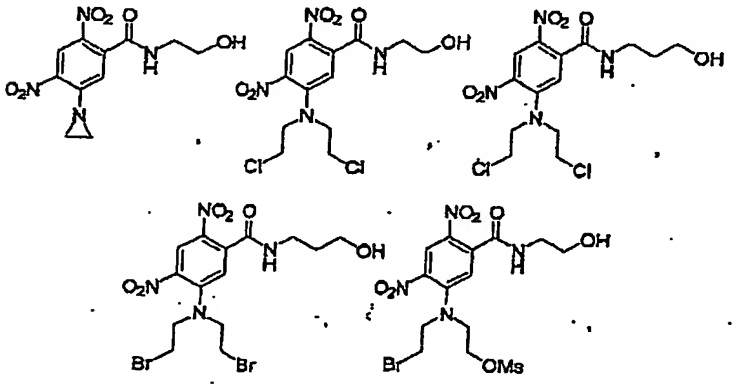
and wherein

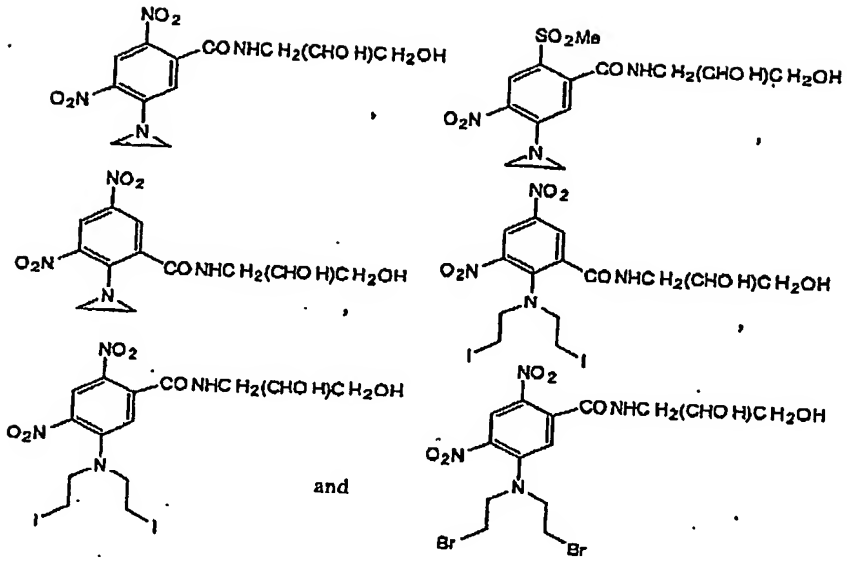
n represents 1 to 6

Z represents $-NO_2$, -halogen, $-CN$, $-CF_3$ or $-SO_2Me$; and

where each W is independently selected from halogen or $-OSO_2Me$

and pharmaceutically acceptable salts and derivatives thereof with the proviso that when Z represents NO_2 and Y represents $N(CH_2CH_2Cl)_2$, X and R together cannot represent $-CONHCH_2(CHOH)CH_2-$ and with the further proviso that the following compounds





and

are excluded.

Preferably the compound of Formula (II) is selected from the following:

N-(2-Hydroxyethyl)-5-[bis(2-bromoethyl)amino]-2,4- dinitrobenzamide;

N-(4-Hydroxybutyl)-5-[bis(2-bromoethyl)amino]-2,4-dinitrobenzamide;

N-(5-Hydroxypentyl)-5-[bis(2-bromoethyl)amino]-2,4-dinitrobenzamide;

N-(6-Hydroxyhexyl)-5-[bis(2-bromoethyl)amino]-2,4-dinitrobenzamide;

5-[Bis(2-bromoethyl)amino]-N-(2-hydroxyethyl)-4-(methylsulfonyl)-2-nitrobenzamide;

2[(2-Bromoethyl)-5-[[(3-hydroxypropyl)amino]carbonyl]-2,4-dinitroanilino]ethyl methanesulfonate;

5-[Bis(2-iodoethyl)amino]-N-(2-hydroxyethyl)-2, 4-dinitrobenzamide;

2-[Bis(2-Chloroethyl)amino]-N-(2-hydroxyethyl)-3,5-dinitrobenzamide;

2-[Bis(2-bromoethyl)amino]-N-(2-hydroxyethyl)-3,5-dinitrobenzamide;

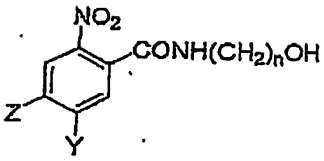
2-[Bis(2-chloroethyl)amino]-N-(3-hydroxypropyl)-3,5-dinitrobenzamide;

2-[Bis(2-bromoethyl)amino]-N-(3-hydroxypropyl)-3,5-dinitrobenzamide;

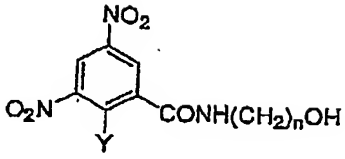
2-[Bis(2-chloroethyl)amino]-N-(4-hydroxybutyl)-3,5-dinitrobenzamide;

2-[Bis(2-bromoethyl)amino]-N-(4-hydroxybutyl)-3,5-dinitrobenzamide;

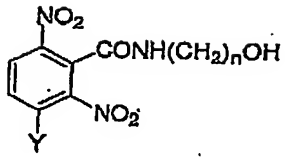
2-[Bis(2-chloroethyl)amino]-N-(5-hydroxypentyl)-3,5-dinitrobenzamide;



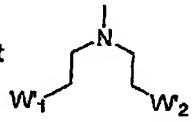
(IIa')



(IIb')



(IIc')

wherein Y may represent 

wherein W'_1 and W'_2 are each halogen;

with an effective amount of silver methanesulfonate (AgOMs) in a solvent to give a compound of formulae (IIa), (IIb) or (IIc) defined above.

It is to be appreciated that in the method defined immediately above where W'_1 and W'_2 are either iodine and/or bromine that the iodine and/or bromine can be partially or completely substituted with $-OSO_2Me$. In the situation where either or both of W'_1 and W'_2 represent chlorine, the chlorine is inert and cannot be substituted with $-OSO_2Me$.

Preferably the solvent is selected from MeCN or other polar non-protic solvent.

In a fifth aspect there is provided a method of preparing a compound of formulae (Ia), (Ib) or (Ic)

Preferably the nitroreductase enzyme is encoded for by the nfsB gene of *E.coli* or by orthologous genes in *Clostridia* species.

Preferably the method includes the further step of irradiating the tumour cells.

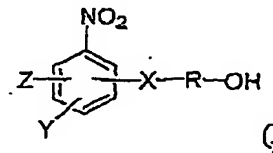
It is to be appreciated that with ADEPT it may be necessary to supply a reducing co-factor, because these may not be present in significant concentrations outside cells. It is envisaged that a synthetic co-factor could be used to stimulate activation of the pro-drug by the likes of an intracellular enzyme. The same issue does not arise with GDEPT because there are several intracellular reducing co-factors such as the likes of NADH and NADPH in significant concentrations.

In a ninth aspect, the present invention provides a method of providing anticancer treatment, wherein a compound of Formula (I) as defined above is administered in an amount to a subject.

Preferably the amount of said method is between about 20% to 100% of the maximum tolerated dose of said subject.

Preferably, the method includes the further step of applying radiation or chemotherapeutic agents to the tumour cells.

In a tenth aspect of the present invention, there is provided a method of cell ablation utilising at least one nitroreductase enzyme, wherein the method includes the step of administering a compound of Formula (I) as defined a above or a compound of Formula (II)



(II)

wherein:

X represents at any available ring position –CONH-, -SO_2NH-, -O-, -CH_2-, -NHCO- or

$-NHSO_2-$;

Y represents at any available ring position -N-aziridinyl, $-N(CH_2CH_2W)_2$ or $-N(CH_2CHMeW)_2$, where each W is independently selected from halogen or $-OSO_2Me$;

Z represents at any available ring position $-NO_2$, -halogen, -CN, $-CF_3$ or $-SO_2Me$;

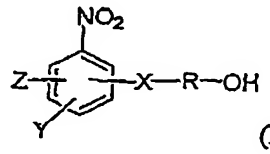
R represents a lower C_{1-6} alkyl optionally substituted with one or more groups including hydroxy, amino and N-oxides therefrom or dialkylamino and N-oxides therefrom; and pharmaceutically acceptable salts and derivatives thereof, or a mixture thereof in an effective amount to ablate cells, wherein said cells express at least one nitroreductase enzyme.

Preferably the nitroreductase enzyme is encoded for by the nfsB gene in *E. coli* or by orthologous genes in *Clostridia* species.

Preferably, the cells that are targeted for ablation are tumor cells in tissue in a subject.

Preferably, the method of cell ablation utilising at least one nitroreductase enzyme is delivered by either ADEPT or GDEPT technology.

In an eleventh aspect of the present invention there is provided a pharmaceutical composition including a therapeutically effective amount of a compound of Formula (I) or a compound of Formula (II)



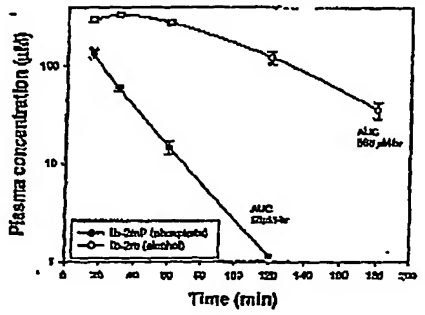
(II)

wherein:

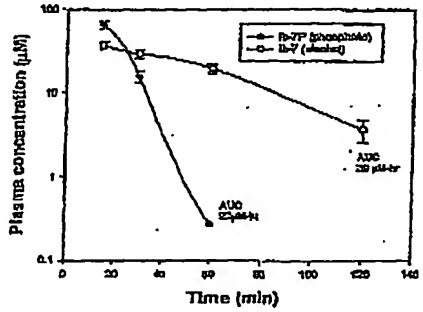
X represents at any available ring position –CONH-, $-SO_2NH-$, -O-, $-CH_2-$, -NHCO- or $-NHSO_2-$;

The following graph shows the pharmacokinetics of the phosphate esters Ib-2mP, Ib-7P, Ib-12P and Ic-12P following administration to female CD-1 nude mice by intraperitoneal injection at a dose corresponding to 75% of the maximum tolerated dose. Monosodium salts of the compounds were dissolved in phosphate buffered saline, pH 7.4, with addition of one equivalent of sodium bicarbonate. Serial blood samples were obtained by small tail vein bleeds, and 10 ul of plasma were prepared from each. Proteins were precipitated by addition of 3 volumes of methanol, and concentrations of the phosphate esters and corresponding alcohols were determined by HPLC using either UV or mass spectrometry detection.

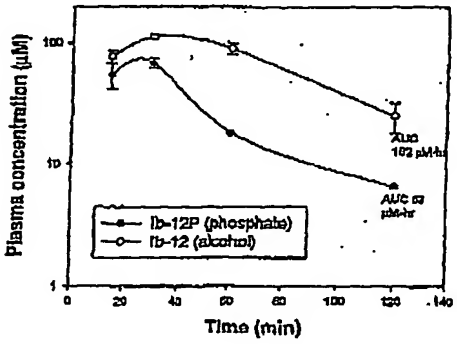
Cmpd Ib-2mP (1.333 mmol/kg, i.p., female CD-1 nude mice)



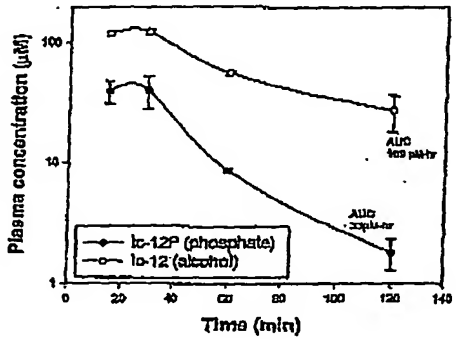
Cmpd Ib-7P (0.562 mmol/kg, i.p., female CD-1 nude mice)



Cmpd Ib-12P (1 mmol/kg, i.p., female CD-1 nude mice)



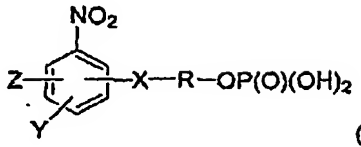
Cmpd Ic-12P (1.33 mmol/kg, i.p., female CD-1 nude mice)



The data shows that the phosphate esters are converted efficiently to the corresponding alcohols in mice. The alcohols are the species activated by nitroreduction in hypoxic, or NTR-expressing, cells.

Amended Claims

1. A phosphate compound of Formula (I)



(I)

wherein:

X represents at any available ring position –CONH-, $-SO_2NH-$, -O-, $-CH_2-$, -NHCO- or $-NHSO_2-$;

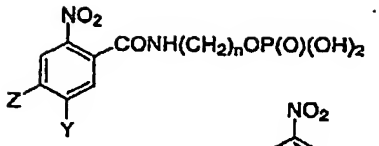
R represents a lower C_{1-6} alkyl optionally substituted with one or more groups including hydroxy, amino and N-oxides therefrom or dialkylamino and N-oxides therefrom;

Y represents at any available ring position -N-aziridinyl, $-N(CH_2CH_2W)_2$ or $-N(CH_2CHMeW)_2$, where each W is independently selected from halogen or $-OSO_2Me$.

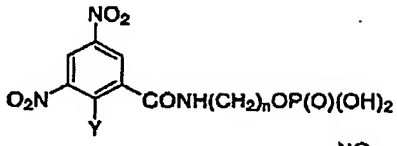
Z represents at any available ring position $-NO_2$, -halogen, -CN, $-CF_3$ or $-SO_2Me$;

and pharmaceutically acceptable salts and derivatives thereof.

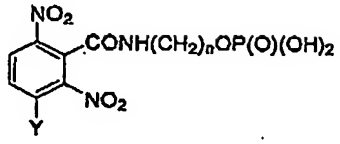
2. A phosphate compound of Formula (I) as claimed in claim 1 which is selected from a compound represented by formulae (Ia), (Ib) or (Ic)



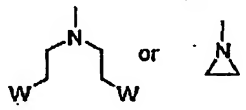
(Ia)



(Ib)



(Ic)

wherein Y represents 

or

and wherein

n represents 1 to 6

Z represents $-NO_2$, -halogen, -CN, $-CF_3$ or $-SO_2Me$; and

where each W is independently selected from halogen or $-OSO_2Me$

and pharmaceutically acceptable salts and derivatives thereof.

3. The phosphate compound of Formula (I) as claimed in claim 1 or claim 2 which is selected from:

2-[[2-[Bis(2-bromoethyl)amino]-3,5-dinitrobenzoyl]amino]ethyl dihydrogen phosphate;

3-[[5-[Bis(2-chloroethyl)amino]-2,4-dinitrobenzoyl]amino]propyl dihydrogen phosphate;

3-[[5-[Bis(2-bromoethyl)amino]-2,4-dinitrobenzoyl]amino]propyl dihydrogen phosphate;

2-[[2-[Bis(2-chloroethyl)amino]-3,5-dinitrobenzoyl]amino]ethyl dihydrogen phosphate;

2-[(2-Chloroethyl)-2,4-dinitro-6-[[[2-(phosphonooxy)ethyl]amino]-carbonyl]anilino]ethyl methanesulfonate;

2-({2-[Bis(2-bromopropyl)amino]-3,5-dinitrobenzoyl}amino)ethyl dihydrogen phosphate;

2-[(2-Bromoethyl)-2,4-dinitro-6-[[[2-(phosphonooxy)ethyl]amino]-carbonyl]anilino]ethyl methanesulfonate;

2-[[2-[Bis(2-iodoethyl)amino]-3,5-dinitrobenzoyl]amino]ethyl dihydrogen phosphate;

2-[(2-Iodoethyl)-2,4-dinitro-6-({[2-(phosphonooxy)ethyl]amino}carbonyl)-anilino]ethyl methanesulfonate;

2-[(2-Chloroethyl)-2,4-dinitro-3-[[[3-(phosphonooxy)propyl]amino]-carbonyl]anilino]ethyl methanesulfonate;

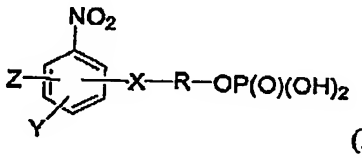
3-({3-[Bis(2-bromoethyl)amino]-2,6-dinitrobenzoyl}amino)propyl dihydrogen phosphate;

2-[(2-Bromoethyl)-2,4-dinitro-3-[[[2-(phosphonooxy)ethyl]amino]-carbonyl]anilino]ethyl methanesulfonate;

2-[(2-Bromoethyl)-2,4-dinitro-3-[[[3-(phosphonooxy)propyl]amino]-carbonyl]anilino]ethyl methanesulfonate; and

2-[(2-Iodoethyl)-2,4-dinitro-3-[[[3-(phosphonooxy)propyl]amino]-carbonyl]anilino]ethyl methanesulfonate.

4. A method of preparing a phosphate represented by the general formula (I);



(I)

wherein:

X represents at any available ring position –CONH-, $-SO_2NH-$, -O-, $-CH_2-$, -NHCO- or $-NHSO_2-$;

R represents a lower C_{1-6} alkyl optionally substituted with one or more groups including hydroxy, amino and N-oxides therefrom or dialkylamino and N-oxides therefrom;

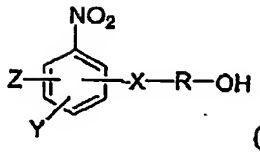
Y represents at any available ring position -N-aziridinyl or $-N(CH_2CH_2W)_2$, where each W is independently selected from halogen or $-OSO_2Me$;

Z represents at any available ring position $-NO_2$, -halogen, -CN, $-CF_3$ or $-SO_2Me$;

and pharmaceutically acceptable salts and derivatives thereof;

the method including the step of

(i) phosphorylating a compound of formula (II)



(II)

wherein:

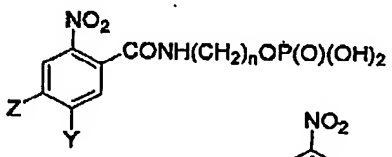
X represents at any available ring position –CONH-, -SO_2NH-, -O-, -CH_2-, -NHCO- or -$NHSO_2$-;

Y represents at any available ring position -N-aziridinyl, -$N(CH_2CH_2W)_2$, or -$N(CH_2CH\,MeW)_2$ where each W is independently selected from halogen or -OSO_2Me;

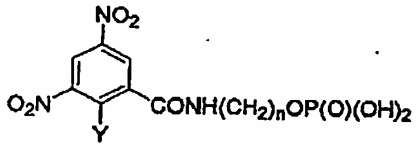
Z represents at any available ring position -NO_2, -halogen, -CN, -CF_3 or -SO_2Me; and

R represents a lower C_{1-6} alkyl optionally substituted with one or more groups including hydroxy, amino and N-oxides therefrom or dialkylamino and N-oxides therefrom.

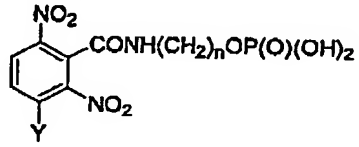
5. A method of preparing a compound of formulae (Ia), (Ib) or (Ic)



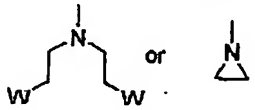
(Ia)



(Ib)



(Ic)

wherein Y may represent  or

and wherein

n represents 1 to 6

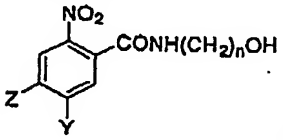
Z represents -NO_2, -halogen, -CN, -CF_3 or -SO_2Me; and

where each W is independently selected from halogen or -OSO_2Me

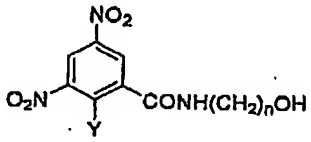
and pharmaceutically acceptable salts and derivatives thereof

the method including the step of

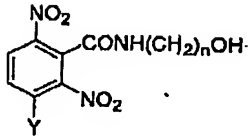
phosphorylating a compound represented by formulae (IIa), (IIb) or (IIc)



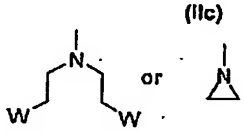
(IIa)



(IIb)



(IIc)

wherein Y represents 

and wherein

n represents 1 to 6

Z represents $-NO_2$, -halogen, -CN, $-CF_3$ or $-SO_2Me$; and

where each W is independently selected from halogen or $-OSO_2Me$

and pharmaceutically acceptable salts and derivatives.

6. A compound of formula (I) when obtained by the method defined in claim 4.

7. A compound of formula (Ia), (Ib) or (Ic) when obtained by the method defined in claim 5.

8. A method of anticancer treatment including the step of administering an amount of a compound of Formula (I) as defined above in any one of claims 1 to 3 to a subject.

9. A method of killing hypoxic cells in a tumour including the step of administering an amount of a compound of Formula (I) as defined above in any one of claims 1 to 3 to a subject with the tumour.

10. The method as claimed in claim 8 or claim 9 including the further step of applying irradiation or one or more chemotherapeutic agents to the subject.

11. The method as claimed in any one of claims 8 to 10 wherein the subject is a human.

12. The method as claimed in any one of claims 8 to 11 wherein the amount administered is between about 20% to 100% of the maximum tolerated dose of the subject.

13. A method of cell ablation utilising at least one nitroreductase enzyme including the step of using a compound of Formula (I) as defined above in any one of claims 1 to 3 in an effective amount to ablate cells which express at least one nitroreductase enzyme.

14. A method of cell ablation utilising at least one nitroreductase enzyme including the step of administering a compound of Formula (I) as defined above in any one of claims 1 to 3 in an effective amount to a subject to ablate cells which express at least one nitroreductase enzyme.

15. The method as claimed in claim 14 wherein the at least one nitroreductase enzyme is encoded for by the nfsB gene of either *E. coli* or by orthologous genes in *Clostridia* species.

16. The method as claimed in claim 14 or claim 15 wherein the cells that express the at least one nitroreductase enzyme are tumour cells in tissue in the subject.

17. The method as claimed in any one of claims 14 to 16 wherein the cell ablation is achieved through GDEPT (gene-directed enzyme-prodrug therapy).

18. The method as claimed in any one of claims 14 to 17 wherein the cell ablation is achieved through ADEPT (antibody-directed enzyme-prodrug therapy).

19. The method as claimed in any one of claims 14 to 18 wherein the cells are mammalian.

20. The method as claimed in any one of claims 14 to 19 wherein the amount administered is between about 20% to 100% of the maximum tolerated dose of the subject.

21. The method as claimed in any one of claims 14 to 20 including the further step of applying irradiation or one or more chemotherapeutic agents to the subject.

22. A pharmaceutical composition including a therapeutically effective amount of a compound of Formula (I) as defined in any one of claims 1 to 3 and a pharmaceutically acceptable excipient, adjuvant, carrier, buffer or stabiliser

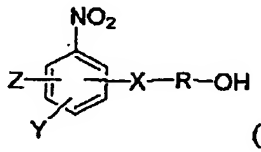
23. The use in the manufacture of a medicament of an effective amount of a compound of Formula (I) as defined in any one of claims 1 to 3 to treat cancer in a subject.

24. The use as claimed in claim 23 wherein the medicament is further adapted for use in cell ablation in conjunction with at least one nitroreductase enzyme including GDEPT (gene-directed enzyme-prodrug therapy) or ADEPT (antibody-directed enzyme therapy).

25. The use as claimed in 24 wherein the at least one nitroreductase enzyme is encoded for by the nfsB gene of either *E. coli* or by orthologous genes in *Clostridia* species.

26. The use as claimed in any one of claims 23 to 25 wherein the medicament is adapted for a mammalian subject.

27. An alcohol compound of Formula (II)



(II)

wherein:

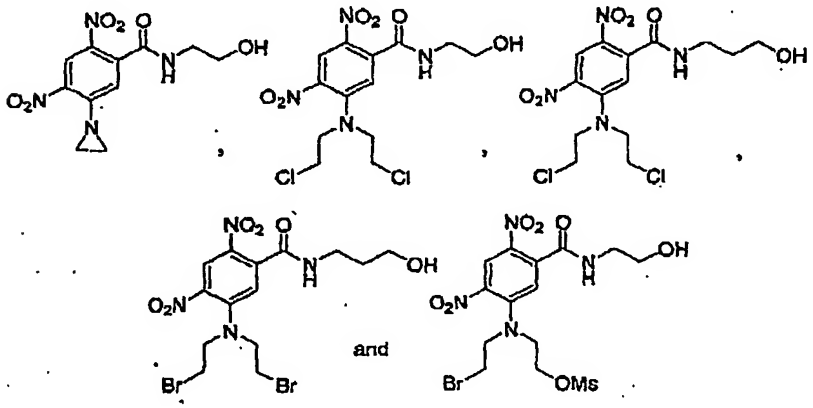
X represents at any available ring position –CONH-, $-SO_2NH-$, -O-, $-CH_2-$, -NHCO- or $-NHSO_2-$;

Y represents at any available ring position -N-aziridinyl, $-N(CH_2CH_2W)_2$, or $-N(CH_2CHMeW)_2$ where each W is independently selected from halogen or $-OSO_2Me$;

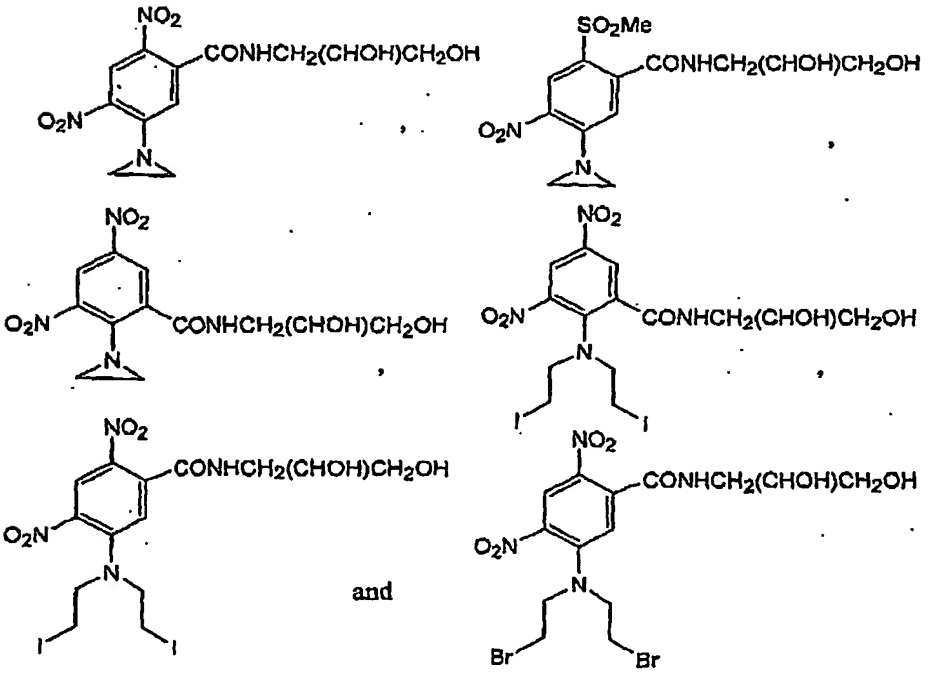
Z represents at any available ring position $-NO_2$, -halogen, -CN, $-CF_3$ or $-SO_2Me$;

R represents a lower C_{1-6} alkyl optionally substituted with one or more groups including hydroxy, amino and N-oxides therefrom or dialkylamino and N-oxides therefrom; and pharmaceutically acceptable salts and derivatives thereof, with the proviso that

when Z represents NO_2 and Y represents $N(CH_2CH_2Cl)_2$, X and R together cannot represent -$CONHCH_2(CHOH)CH_2$- and with the further proviso that the following compounds



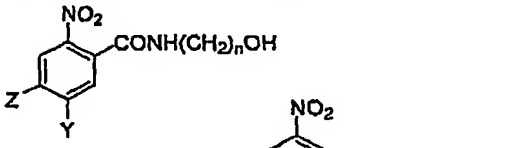
and



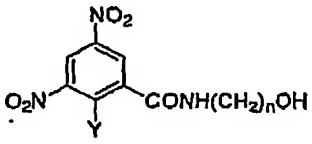
and

are excluded.

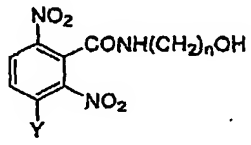
28. The alcohol compound of Formula (II) as claimed in claim 27 selected from a compound represented by formulae (IIa), (IIb) or (IIc)



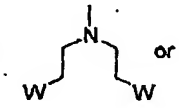
(IIa)



(IIb)



(IIc)

wherein Y may represent  or 

and wherein

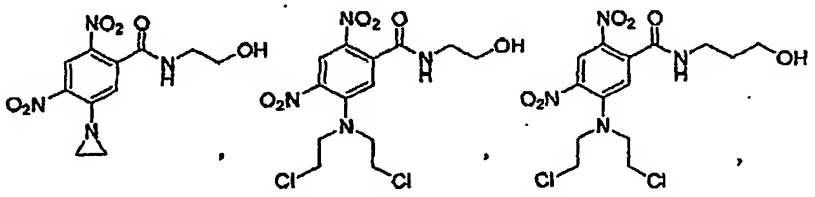
n represents 1 to 6

Z represents $-NO_2$, -halogen, -CN, $-CF_3$ or $-SO_2Me$; and

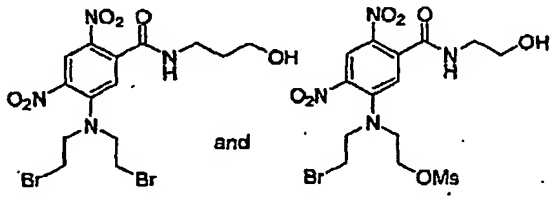
where each W is independently selected from halogen or $-OSO_2Me$

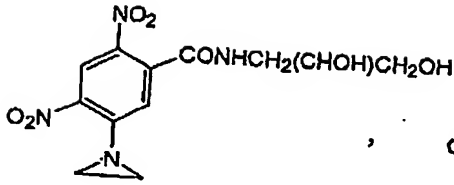
and pharmaceutically acceptable salts and derivatives thereof with the proviso that

when Z represents NO_2 and Y represents $N(CH_2CH_2Cl)_2$, X and R together cannot represent - $CONHCH_2(CHOH)CH_2$- and with the further proviso that the following compounds



and





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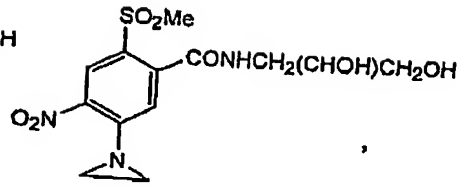

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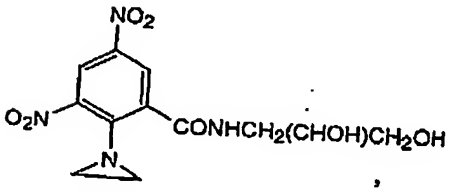

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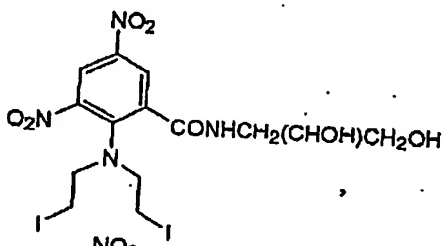

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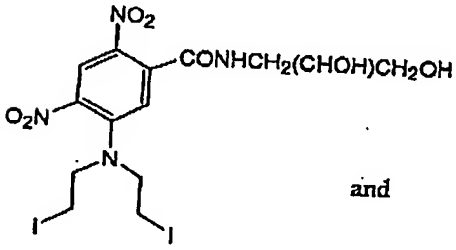
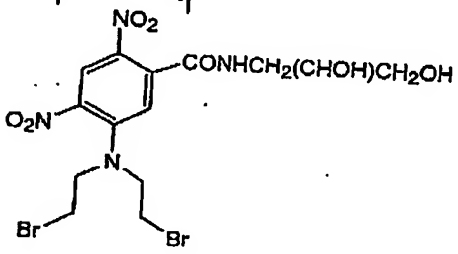


and



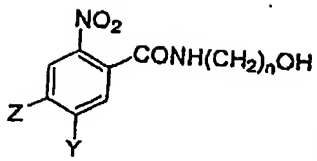
are excluded.

29. The alcohol compound of Formula (II) selected from a compound of Formula (IIb) or (IIc) as defined in claim 28.

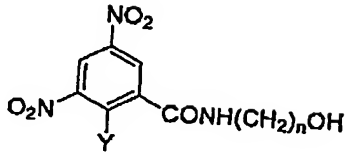
30. The alcohol compound of Formula (II) as defined in claim 28 or claim 29 selected from:
N-(2-Hydroxyethyl)-5-[bis(2-bromoethyl)amino]-2,4- dinitrobenzamide;
N-(4-Hydroxybutyl)-5-[bis(2-bromoethyl)amino]-2,4-dinitrobenzamide;
N-(5-Hydroxypentyl)-5-[bis(2-bromoethyl)amino]-2,4-dinitrobenzamide;
N-(6-Hydroxyhexyl)-5-[bis(2-bromoethyl)amino]-2,4-dinitrobenzamide;
5-[Bis(2-bromoethyl)amino]-N-(2-hydroxyethyl)-4-(methylsulfonyl)-2-nitrobenzamide;
2[(2-Bromoethyl)-5-[[(3-hydroxypropyl)amino]carbonyl]-2,4-dinitroanilino]ethyl methanesulfonate;
5-[Bis(2-iodoethyl)amino]-N-(2-hydroxyethyl)-2, 4-dinitrobenzamide;
2-[Bis(2-Chloroethyl)amino]-N-(2-hydroxyethyl)-3,5-dinitrobenzamide;
2-[Bis(2-bromoethyl)amino]-N-(2-hydroxyethyl)-3,5-dinitrobenzamide;
2-[Bis(2-chloroethyl)amino]-N-(3-hydroxypropyl)-3,5-dinitrobenzamide;
2-[Bis(2-bromoethyl)amino]-N-(3-hydroxypropyl)-3,5-dinitrobenzamide;

2-[Bis(2-chloroethyl)amino]-N-(4-hydroxybutyl)-3,5-dinitrobenzamide;
2-[Bis(2-bromoethyl)amino]-N-(4-hydroxybutyl)-3,5-dinitrobenzamide;
2-[Bis(2-chloroethyl)amino]-N-(5-hydroxypentyl)-3,5-dinitrobenzamide;
2-[Bis(2-bromoethyl)amino]-N-(5-hydroxypentyl)-3,5-dinitrobenzamide;
2-[Bis(2-chloroethyl)amino]-N-(6-hydroxyhexyl)-3,5-dinitrobenzamide;
2-[Bis(2-bromoethyl)amino]-N-(6-hydroxyhexyl)-3,5-dinitrobenzamide;
2-[Bis(2-bromopropyl)amino]-N-(2-hydroxyethyl)-3,5-dinitrobenzamide;
2-((2-Bromoethyl)-2-{[(2-hydroxypropyl)amino]carbonyl}-4,6-dinitroanilino)ethyl methanesulfonate;
2-((2-Bromoethyl)-2-{[(2-hydroxyethyl)amino]carbonyl}-4,6-dinitroanilino)ethyl methanesulfonate;
2-((2-Chloroethyl)-2-{[(2-hydroxyethyl)amino]carbonyl}-4,6-dinitroanilino)ethyl methanesulfonate;
2-[Bis(2-iodoethyl)amino]-N-(2-hydroxyethyl)-3,5-dinitrobenzamide;
2-((2-Iodoethyl)-2-{[(2-hydroxyethyl)amino]carbonyl}-4,6-dinitroanilino)ethyl methanesulfonate;
3-[Bis(2-bromoethyl)amino]-N-(2-hydroxyethyl)-2,6-dinitrobenzamide;
2-((2-Bromoethyl)-3-{[(2-hydroxyethyl)amino]carbonyl}-2,4-dinitroanilino)ethyl methanesulfonate;
3-[Bis(2-bromoethyl)amino]-N-(3-hydroxypropyl)-2,6-dinitrobenzamide;
2-((2-bromoethyl)-3-{[(3-hydroxypropyl)amino]carbonyl}-2,4-dinitroanilino)ethyl methanesulfonate;
3-[Bis(2-bromoethyl)amino]-N-(4-hydroxybutyl)-2,6-dinitrobenzamide;
2-((2-Bromoethyl)-3-{[(4-hydroxybutyl)amino]carbonyl}-2,4-dinitroanilino)ethyl methanesulfonate;
2-((2-Chloroethyl)-3-{[(3-hydroxypropyl)amino]carbonyl}-2,4-dinitroanilino)ethyl methanesulfonate; and
2-((2-Iodoethyl)-3-{[(3-hydroxypropyl)amino]carbonyl}-2,4-dinitroanilino)ethyl methanesulfonate.

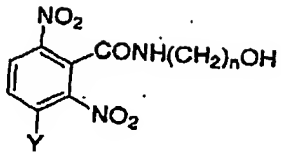
31. A method of preparing a compound of formulae (IIa), (IIb) or (IIc)



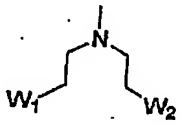
(IIa)



(IIb)



(IIc)

wherein Y may represent 

and wherein

n represents 1 to 6

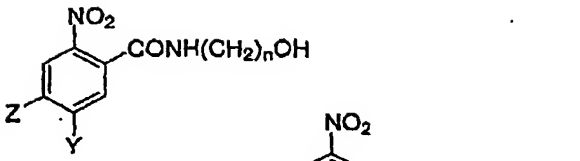
Z represents $-NO_2$, -halogen, -CN, $-CF_3$ or $-SO_2Me$; and

where W_1 is halogen and W_2 is $-OSO_2Me$

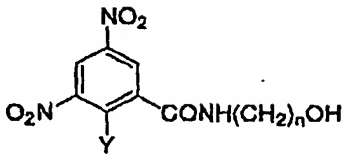
and pharmaceutically acceptable salts and derivatives thereof;

the method including the step of

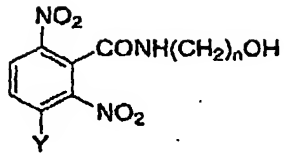
reacting a compound of formulae (IIa'), (IIb') or (IIc') optionally with heating



(IIa')

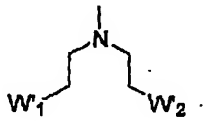


(IIb')



(IIc')

wherein Y may represent



wherein W'_1 and W'_2 are each halogen;

with an effective amount of silver methanesulfonate (AgOMs) in a solvent to give a compound of formulae (IIa), (IIb) or (IIc) defined above in this claim.

32. The method as claimed in claim 31 wherein the solvent is selected from MeCN or other polar non-protic solvent.

33. A compound of formula (IIa), (IIb) or (IIc) obtained by the method defined in claim 31 or claim 33.

34. A method of anticancer treatment including the step of administering an amount of a compound of Formula (II) as defined in claim 27 to a subject.

35. A method of killing hypoxic cells in a tumour including the step of administering an amount of a compound of Formula (II) as defined in claim 27 to a subject with the tumour.

36. The method as claimed in claim 34 or claim 35 including the further step of applying irradiation or one or more chemotherapeutic agents to the subject.

37. The method as claimed in any one of claims 34 to 36 wherein the subject is a human.

38. A method of cell ablation utilising at least one nitroreductase enzyme including the step of using a compound of Formula (II) as defined in claim 27 in an effective amount to ablate cells which express at least one nitroreductase enzyme.

39. A method of cell ablation utilising at least one nitroreductase enzyme including the step of administering a compound of Formula (II) as defined in claim 27 in an effective amount to a subject to ablate cells which express at least one nitroreductase enzyme.

40. The method as claimed in claim 39 wherein the at least one nitroreductase enzyme is encoded for by the nfsB gene of either *E. coli* or by orthologous genes in *Clostridia* species.

41. The method as claimed in claim 39 or claim 40 wherein the cells that express the at least one nitroreductase enzyme are tumour cells in tissue in the subject.

42. The method as claimed in any one of claims 39 to 41 wherein the cell ablation is achieved through GDEPT (gene-directed enzyme-prodrug therapy).

43. The method as claimed in any one of claims 39 to 41 wherein the cell ablation is achieved through ADEPT (antibody-directed enzyme-prodrug therapy).

44. The method as claimed in any one of claims 39 to 43 wherein the cells are mammalian.

45. The method as claimed in any one of claims 39 to 44 including the further step of applying irradiation or one or more chemotherapeutic agents to the subject.

46. A pharmaceutical composition including a therapeutically effective amount of a compound of Formula (II) as claimed in claim 27 and a pharmaceutically acceptable excipient, adjuvant, carrier, buffer or stabiliser.

47. The use in the manufacture of a medicament of an effective amount of a compound of Formula (II) as claimed in claim 27 as an anticancer agent in a subject.

48. The use as claimed in claim 47 wherein the medicament is further adapted for use in cell ablation in conjunction with at least one nitroreductase enzyme including GDEPT (gene-directed enzyme-prodrug therapy) or ADEPT (antibody-directed enzyme therapy).

49. The use as claimed in claim 48 wherein the at least one nitroreductase enzyme is encoded for by the nfsB gene of either *E. coli* or by orthologous genes in *Clostridia* species.

50. The use as claimed in any one of claims 47 to 49 wherein the medicament is adapted for a mammalian subject.